LIGHT-INHIBITION OF FLASHING IN THE FIREFLY PHOTURIS MISSOURIENSIS [1]

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Recent investigations of photic inhibition of flashing in the firefly *Luciola* (Magni, 1967; Brunelli *et al.*, 1968a, 1968b) have confirmed the phenomenon of inhibition *via* the visual system reported by Case and Buck (1963), who suggested that the observed inhibition was largely of central origin although they did observe, under conditions of continuous glowing, "unflashes"—dimming of continuous glowing with flash kinetics—upon electrical excitation of the light organ, raising the possibility of a peripheral mechanism. In *Luciola*, however, there appears to be a possibly humorally linked inhibition somehow involving the testis, as evidenced by transferral of inhibition across a saline bridge from a light-inhibited firefly and by disappearance of this phenomenon upon castration of the light-inhibited individual. The inhibitory process in *Luciola* was further shown to have a peripheral element by inhibition of flashes driven by electrical stimulation of the light organ upon illumination of the eyes (Magni, 1967).

Since peripheral inhibition has not readily been demonstrated in insects it is most desirable to determine the extent to which the *Luciola* inhibitory system is present in other species of fireflies.

The present report represents an unsuccessful attempt to detect peripheral inhibition in *Photuris missouriensis*. It provides additional information regarding the nature of the inhibition, originally reported in *Photuris* by Case and Buck (1963), and which seems to be wholly a central nervous system process acting on an exclusively excitatory peripheral light organ innervation.

Materials and Methods

Fireflies were obtained principally from collectors in Kansas and Iowa, for whose supervision we are most indebted to Dr. Katherine Smalley of Kansas State College, Emporia. Until use, the fireflies were fed sucrose solution and kept at room temperature or stored at 10° C. Luminescence was detected *via* fiber optic light guides and 931A photomultipliers and recorded on an Offner Dynograph and photographically from a Tektronix 533 oscilloscope. Electrical excitation was delivered from constant-current stimulators or pulse generators. Light stimuli were delivered *via* a fiber optic guide from an incandescent or xenon arc lamp.

Localization of light stimuli was insured by mounting insects with their heads projecting into a hole in a black plastic block receiving the stimulating light guide and sealing the aperture with a mixture of powered graphite in Vaseline. Light

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TABLE 1

Time required to photically inhibit spontaneous flashing

Illumination lead time (msec.)	Extent of inhibition of expected following flashes (1.0 = normal; 0.0 = no flash)					
0–40	1.0	1.0	1.0	1.0	1.0	
41–80	1.0	1.0	1.0			
81–120	1.0	0.6	1.0	1.0	0.8	
121–160	0.6	0.0	1.0	0.0	0.2	1.0
161–200	0.0	0.0	0.0	0.0		
201–240	0.0	0.0	0.0	0.0	0.0	0.0
241–280	0.0	0.0	0.0			
281–960	24 expected flashes, all 0.0					

Illumination 3800 lux. Combined data collected from 3 male *Photuris* by random illumination during sequences of regular spontaneous flashing.

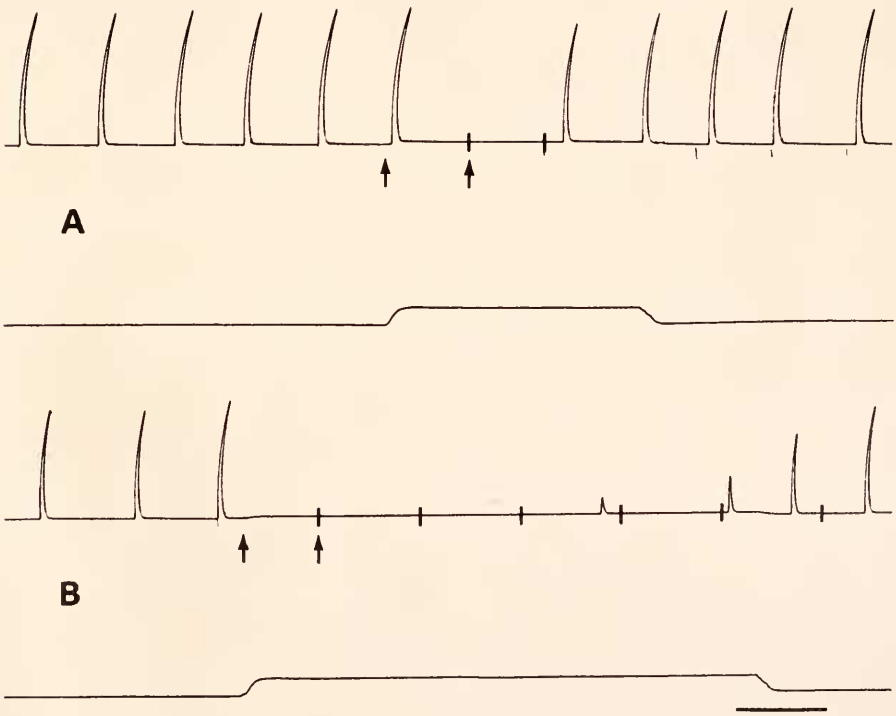

FIGURE 1. Example of records used in determination of latency of photic inhibition *via* eyes of spontaneous flashing *Photuris*. Upper trace in each record registers flashes and lower monitors light source. Latency, the interval between arrows, is measured as time between start of illumination and time of next succeeding expected flash, indicated as vertical bars, as measured from inter-flash interval of several preceding flashes. In 1A, the light goes on insufficiently early to inhibit one flash, completely inhibits the next and possibly partially inhibits and delays a third. Better examples of partial inhibition are shown in 1B. Light stimulus 100 lux, time mark at lower right, 1 second.

sources were calibrated against a Gamma Instruments standard lamp using a Reeder Thermopile and Keithley millimicrovoltmeter. Nor-epinephrine was injected with a Hamilton microliter syringe. The saline of Brunelli *et al.* (1968a) was used for preparing nor-epinephrine solutions and in the saline bridge experiments.

Results

1. *Inhibition of spontaneous flashing*

Interruption of spontaneous flashing by electrical stimulation of the eye (Case and Buck, 1963) or by illumination of the eye (Magni, 1967) was readily confirmed in *Photuris missouriensis*. In this species the specificity of the interruption appears to be sufficient to permit use of the term inhibition in regard to the

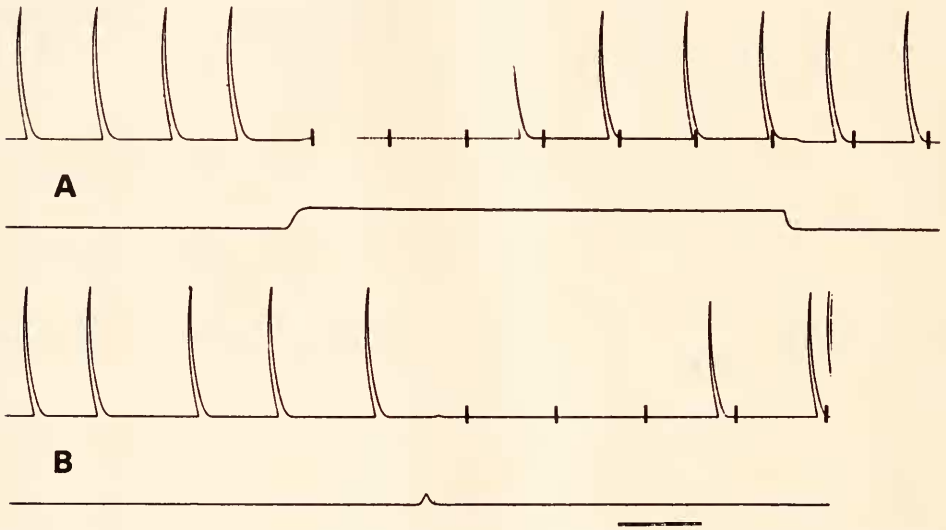

Figure 2. Two sequential photic inhibitions showing that a very brief $\frac{1}{5}$-second, 50-lux flash (2B) can be as effective as a much longer, 100-lux illumination. Upper trace, light organ with some registration of stimulating lamp, which is recorded on lower trace. Time mark, 1 second.

phenomenon. Certainly, the effect is not simply an alarm reaction since *Photuris* responds to injury or disturbance with rapid flashing.

The latency of inhibition is nearly the same for electrical and photic stimulation of the eye. Our most extensive latency measurements were made with light stimuli from either an incandescent or xenon arc lamp. Light stimuli were delivered at random during episodes of regular spontaneous flashing. Latency was estimated as the time from initiation of illumination to the time of the next succeeding modified flash (usually reduced in magnitude) or completely suppressed flash (time of expected occurrence was estimated from the average interflash interval of the preceding series). Results appear in Table I and an example of the records from which the Table was constructed appears as Figure 1. Clearly, illumination at intervals as brief as 160 msec. prior to an expected flash is completely inhibitory. It is

possible to attain inhibition with a lead time of 120 msec. Lead times less than 80 msec. do not inhibit the immediately subsequent flash while they do inhibit the next and following flashes. These observations are not unexpected since Case and Buck (1963) have shown the minimum latency for excitation of luminescence by brain stimulation in *Photuris* to be 120–150 msec.

2. Effects of light intensity and duration on inhibition of spontaneous flashes

These effects are most puzzling since there appears to be a triggering effect of light superimposed upon a graded response. Triggering is illustrated in Figure 2 where an extremely brief illumination of not more than $\frac{1}{5}$th second with maximum intensity of 50 lux resulted in as much inhibition as continuous illumination of 100 lux for six seconds. Light-dark transitions appear often to be more significant than the total illumination delivered because some *Photuris* can be maintained in the inhibited state for a longer period by a train of approximately

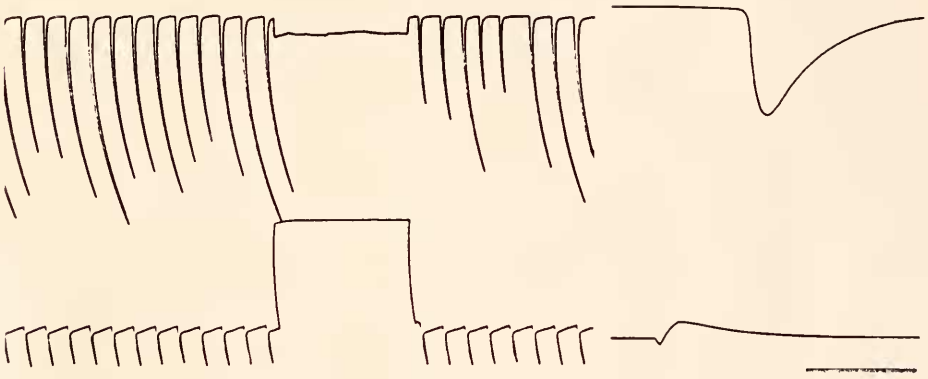

FIGURE 3. Electrical stimulation with paired electrodes in anterior light organ. Flashes recorded as downward deflection of upper trace. Stimulus (5 msec., 5 volts, 1 per second) artifacts and light (1000 lux) on lower trace. Right hand record indicates excitation-flash latency of 136 msec. Time mark at lower right applies to both records: 5 seconds, left; 200 msec. right.

one second on, one second off illuminations than by continuous illumination.

Almost invariably, during all except the most intense illuminations, flashing is reestablished. Typically (Fig. 1b) these escapes are of lower than normal intensity and at a slower frequency than prior to illumination. (The kinetics of the flashes are normal.) There is a marked tendency, however, for flashes subsequent to the first several escapes to occur at the same frequency as prior to illumination and at the same expected time as estimated from pre-illumination flashes, suggesting that illumination does not inhibit a flash pace-making mechanism.

3. Attempts at inhibition of driven flashes

Inhibition of flashes driven at the level of the light organ were unsuccessful in all instances in which the driven flashes were without CNS augmentation. An

example of what would appear to be such inhibition is Figure 3 in which a long series of flashes generated in register with 1/sec. stimulation of the light organ are terminated by illumination. However, the excitation-flash latency (measured oscilloscopically) in this sequence amounts to 136 msec. While this is probably insufficient time to allow facilitative effects *via* the brain, there is clearly sufficient time for reflexive facilitation *via* the abdominal ventral nerve cord. Even if the excitation-flash latency were sufficiently short to preclude CNS effects on the immediately following flash, there is no assurance that a long-term facilatory state built up in the CNS does not affect the flashing sequence. This is illustrated in Figure 4 where a series of voluntary flashes is mixed with driven flashes. Immediate inhibition of the voluntary component occurs upon illumination, along with a gradual diminution of the driven component. We attribute this to an effect of light on the voluntary component, defacilitating the driven flashes. This seems to be confirmed by the events after cessation of illumination in this same experi-

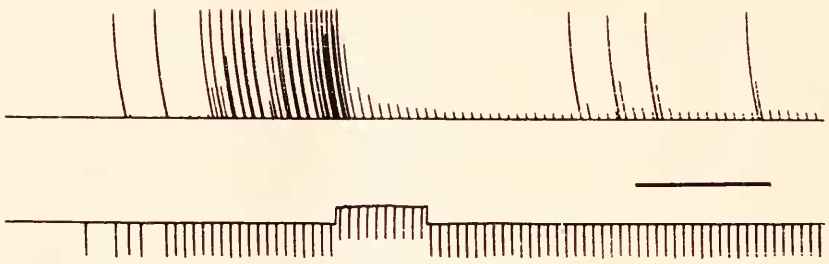

FIGURE 4. Effects of light on voluntary and driven flashes. A series of voluntary flashes, large flashes of uniform intensity, occurs along with electrically driven flashes of irregular intensity. Intensity of driven flashes is seen to depend upon temporal proximity to a previous voluntary flash. Illumination terminates voluntary component with progressive consequent diminution of driven flash magnitude. Upper trace, light organ. Lower trace, stimulus artifact (downward) and illumination artifact (upward). Time mark, 30 secs.

ment in which the driven flashes remain at low ebb until spontaneous flashing resumes. If the level of excitation had been somewhat lower, we presume that the experimental record would have created the impression of complete inhibition of driven flashes by illumination.

4. *Attempts to effect transfer of inhibition from one firefly to another*

The experimental arrangement used by Brunelli *et al.* (1968a) to demonstrate inhibition transfer between individuals in pairs of *Luciola italica* was duplicated in all essential particulars using males of *Photuris missouriensis*. *Photuris* was used in preference to other American species of fireflies because it most readily flashes spontaneously under experimental conditions. In five experiments with good spontaneous flashing it was not possible to demonstrate transfer of inhibition of spontaneous flashing from one *P. missouriensis* to another upon illumination of the eyes of one animal with light (Fig. 5). Since inhibition transfer occurred in the *Luciola* experiments in 8–15 seconds, we attempted to obtain an estimate of the minimal transfer time of an appropriate chemical agent from one insect to

another in our experimental arrangement. Nor-epinephrine was chosen because it rapidly induces glowing and is probably related to the normal synaptic mediator of the firefly light organ (Smalley, 1965). The time required for transfer was measured as the time between induction of glowing, measured with photomultipliers, in one nor-epinephrine-injected member of a saline-linked pair and its non-injected mate. Even where two insects were in direct physical contact *via* the wounds produced by complete severance of their terminal abdominal segments and when one member of the pair had received an injection of nor-epinephrine sufficiently large to induce instantaneous glowing in its own light organ (8 μl. of 10^{-2} M), the minimum transfer time was 68 seconds. One of these experiments is illustrated in Figure 6. In another series of experiments, nor-epinephrine was

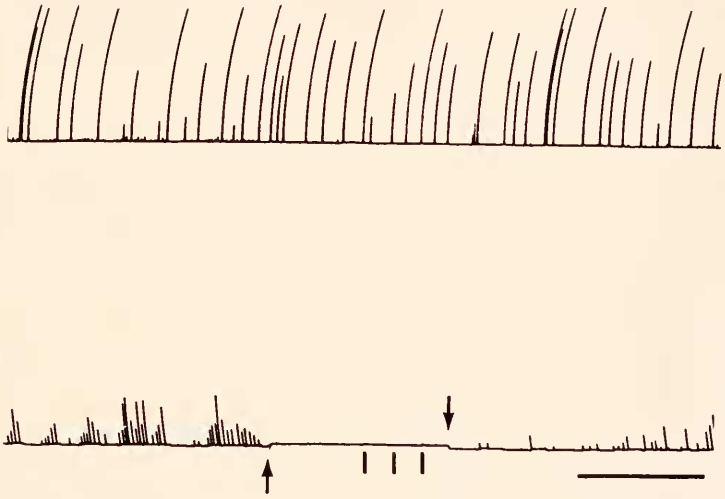

FIGURE 5. Record of an unsuccessful attempt to transfer inhibition from one light-inhibited *Photuris* male, lower trace to another, upper trace, *via* a saline bridge (see text). Light on and off indicated by arrows, 1000 lux. Three minimal escapes, indicated by vertical bars, confirm completeness of inhibition as does the long time required after cessation of illumination to reestablish rapid flashing. Upper trace shows no indication of transfer. Time mark, 30 sec.

introduced into a drop of saline in contact with the hemocoel of the second light organ segment and the time for initiation of glowing in the adjacent segment of the same animal was determined. In these experiments the minimum time required was 30 seconds. Twice this time would, we believe, be comparable to the transfer time between a pair of insects and does, indeed, correspond well with the transfer experiments just described.

5. *The role of the testes in flash inhibition*

Brunelli *et al.* (1968b) report that castration of the light-exposed member of a saline-linked pair made it more difficult to light-inhibit the castrated insect and made transfer of inhibition to the intact member of the pair impossible. We

attempted to further assess the role of the testis by electrical stimulation of a freshly excised pair of *Photuris* testes in a saline drop directly in contact with the light organ tissue of a spontaneously flashing male *Photuris*. Inhibition of flashing was not observed.

Next a more general experiment was conducted to determine if any structures other than CNS and light organ were involved in photic inhibition. The light organ of a spontaneously flashing male *Photuris* was dissected in such a way that its only connection with the remainder of the animal was by way of the ventral nerve cord. In this state, photic inhibition of spontaneous flashing was still obtainable.

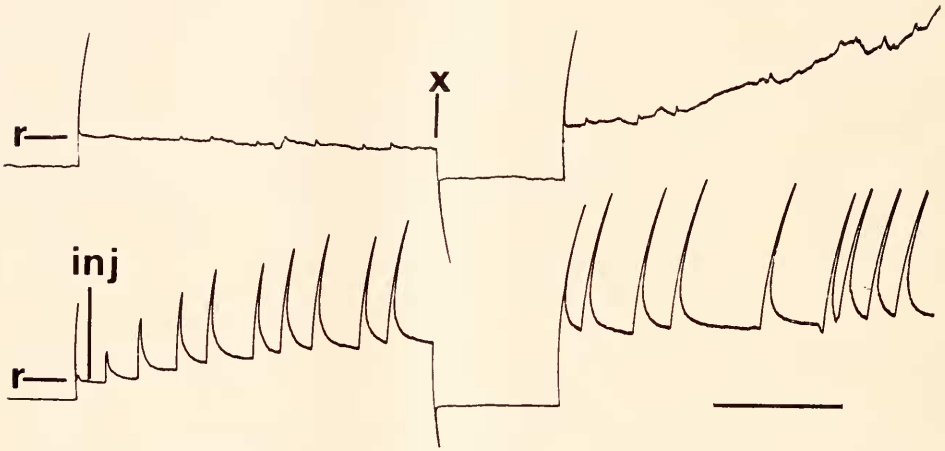

FIGURE 6. Experiment to measure diffusion time of nor-epinephrine between light organs of two fireflies with body cavities joined (see text). Lower trace records rise of luminescence, superimposed upon spontaneous flashing, upon injection at mark from rest luminescent level, r. Upper trace records luminescence from the other member of the pair. Beginning at x, the photomultipliers were turned off during visual confirmation of integrity of saline linkage. By the time recording resumed the second animal had commenced to glow, indicating arrival of nor-epinephrine and leaving the interval terminated at x, 80 seconds, as the minimum diffusion time. Time mark, 30 sec.

DISCUSSION

These data, along with the observations of Case and Buck (1963), Magni (1967) and Brunelli *et al.* (1968a, 1968b), are unfortunately subject to more than one interpretation. When one especially considers the evidence of Carlson (1961) that neural activity exerts a trophic as well as excitatory effect on the lantern, together with the observations concerning the difficulty of "arousing" flashing behavior in fireflies during the day (Case and Buck, 1963), it would seem plausible to propose two flash-suppressing phenomena, both of which are light-initiated. One of these might regulate the circadian cycle of luminescence known to occur at least in *Photinus* (Buck, 1937). The other might effect rapid control of luminescence in response to irregular, brief illuminations during the normally active period. It is plausible to suppose the former might involve a neuro-endocrine link, such as has frequently been observed in insect circadian rhythms while the latter,

because of its rapidity of establishment and of dissipation, might be supposed to be wholly neural in mechanism. The phenomena described in *Luciola* strongly suggest that both mechanisms are present. However, in *Photuris* there does not at present appear to be evidence for any mechanism except the exclusively neural one. In fact, even such phenomena as the "arousal" process and Carlsons' trophic effect might well be simply due to either diminishment of excitation or to a dark-dependent neural excitatory process, rather than peripheral inhibition.

Quenching phenomena, such as described by Case and Buck (1963) have been suggested as possible examples of perhipheral inhibition of steady glowing. Since the mechanism of luminescence control *in vivo* remains unexplained, there is little restriction upon the elaboration of theories concerning peripheral processes such as this electrically driven quenching of denervated light organ glows. By analogy with other bioelectrically activated systems (see, for example, Eckert, 1966) it is plausible that light emission is related to depolarization of photocytes and this concept is strengthened by the K^+ dependence of scintillation in *Photuris* (Carlson, 1967). In glowing light organs, such as those showing the quenching effect, it might be assumed that most photocytes are to some extent depolarized. Electrical excitation of such populations with bipolar electrodes would then probably have hyperpolarizing effects in the vicinity of the anode, resulting in quenching, while cathodal effects would be minimal or non-existent, owing to the already depolarized state of the photocytes.

Resolution of the differences which appear in flash inhibitory mechanisms in *Luciola* and *Photuris* is not readily achieved. Certainly unequivocal photic inhibition of flashes driven at the level of the light organ has not yet been attained in *Photuris*. Their reported occurrence in *Luciola* may well represent a fundamental difference in light organ control in the two species. However, a possibly serious restriction to such interpretation is based on the difficulty of eliminating CNS-mediated facilitatory effects, these assuredly being inhibited by light. In the investigation of *Luciola*, this possibility was eliminated from consideration because the maximal excitatory facilitation time (driven flashes in deganglionated light organs) which is observed is 300 msec. (Buonamici and Magni, 1967, p. 332). Most likely, however, what is critical in experiments on insects with intact CNS and normal sensory input, such as specifically concerns us here, is the central excitatory state which may well remain elevated upon excitation for long periods of time. Thus Dethier *et al.* (1965) report persistence of elevations in central excitatory state of *Phormia*, in response to chemosensory input, of as long as 10 minutes. Such may contribute a sufficient level of facilitation to give false appearance of exclusively directly driven flashes in response to light organ stimulation. Abolishment of central contributions during illumination might then give the appearance of photic inhibition of peripherally driven flashing when, in actuality, the only effect might be simply a reduction in the total level of light organ excitation.

Persistence of photogenic volleys in the ventral nerve cord and light organ of *Luciola* is considered to be an argument for the existence of an inhibitory system acting peripherally to the brain (Magni, 1967). While photogenic volleys have not been recorded in our experiments, the reappearance of flashes in time with pre-inhibited flashes after and during photic inhibition is evidence that these

volleys continue to be generated, or at least that the pacemaker producing them continues to function. The difficulty with assessing the significance of photogenic volley persistence during inhibition lies, of course, in the possibility that their effectiveness in producing flashes may well depend upon other CNS-generated excitatory activity which is not recorded. Certainly the low resolution of all recordings so far made of neural activity associated with flashing does not preclude the presence of small fiber tonic activity which might well be light-inhibited.

Our inability to demonstrate humoral mediation of inhibition or involvement of the testis in inhibition is consistent with the thesis that inhibition is purely a central phenomenon acting on excitatory pathways to the light organ. These negative results can, of course, have no direct bearing on the successful inhibition transfer reported in *Luciola*, although the marked discrepancy of our nor-epinephrine transfer times and the time required to effect inhibition transfer in *Luciola* (over a minute as compared with a few seconds) suggests that factors other than simple diffusion of an inhibitory agent are concerned in the *Luciola* experiments.

Since dim, brief illuminations can bring about effective inhibition (Fig. 2), photic inhibition may play a significant role in flash communication. The flash pattern being of critical importance in communication, photic inhibition might serve to prevent the confusion which might ensue if, for example, two males close together commenced signalling slightly out of phase. Inhibition of the late male by the first flash of the other should prevent signal garbling.

Summary

1. Photic inhibition of spontaneous flashing is demonstrated in *Photuris missouriensis*.

2. The minimum latency of inhibition is between 80 and 160 msec. Inhibition is expressed as completely missed or diminished intensity flashes. There is a tendency for the flashes appearing during inhibition to be later than expected but generally flashing returns to pre-inhibitory timing, suggesting continued operation of a flash pacemaker. The extent of inhibition was found to be light intensity dependent above minimal intensities at which on-off triggering effects are dominant.

3. Direct excitation of the light organ could not be inhibited *via* the visual system.

4. Transfer of inhibition from one *Photuris* to another, *via* saline bridging their body cavities, could not be accomplished.

5. These results, arguing for a purely central mechanism of inhibition in *Photuris*, are compared with those of Magni and Brunelli *et al.*, who propose both central and peripheral inhibition of flashing in *Luciola*.

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